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Research conducted under this grant has demonstrated that lipid content and composition of diatoms varied greatly during the growth cycle. However, more variation was found over a 24 hour period. A portion of the lipid content response was found to be entrained with the light/dark regime. However, the data also suggested that a portion of the lipid cycling was not entrained with the light/dark regime, but may be under control of other biological rhythms. Preliminary toxicant exposure experiments have suggested that the time of day when algae are exposed to toxicants may alter physiological responses to the toxicant.

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INFLUENCE OF LIPID COMPOSITION IN AMPLIFYING OR AMELIORATING  
TOXICANT EFFECTS ON PHYTOPLANKTON

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## PROJECT RATIONALE

The rationale for this project is that lipid quantity and quality may vary with environmental parameters and this in turn may lead to changes in the toxicity of lipophilic compounds to phytoplankton. This hypothesis is based on observations in our laboratory that in the diatom Cyclotella meneghiniana, exposure to isomers of trichlorobenzene produced physiological responses other than those that would be predicted by chemical reactivity, and these results appeared to be correlated with the timing of the exposure to the lipophilic compounds. The data further suggested that the most likely reason for the changes in the toxicity was the lipid content, which appeared to be variable with the light cycle.

With this background in mind, we designed series of experiments to determine the role of environmental factors such as light cycle and temperature on the production of lipids in diatoms, and to determine if lipid content and quality can affect the toxicity of lipophilic compounds. The ultimate goal is to assess the role of physiological mechanisms in ameliorating or amplifying toxicant effects and to determine if certain meteorological or environmental conditions are more conducive to bioaccumulation of lipophilic toxicants.

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## STUDY DESIGN

Three experimental diatoms were chosen for study; Cyclotella meneghiniana, Melosira varians, and Stephanodiscus binderanus. Cyclotella was chosen because it was the experimental organism that was used previously in the studies with trichlorobenzene isomers. It is a small centric diatom, approximately 4  $\mu\text{m}$  in diameter. We have also studied this organism extensively in heavy metal studies, so we have a wealth of information on toxicity response. Stephanodiscus and Melosira are also centric diatoms, but they are quite a bit larger and form chains. It is believed that Melosira may be a predominant food source for benthic animals, and we are ultimately interested in the transfer of lipophilic chemicals to higher trophic levels. Both of these larger diatoms also form resting cells, and during both formation and rejuvenation of resting cells, copious lipid accumulation is observed.

Our approach during the first year of the project has been to determine lipid content of the three diatoms grown in batch culture throughout their growth cycle and to document the 24 hour change in lipid content during logarithmic phase growth under given light regimes and temperatures.

## MATERIALS AND METHODS

For growth curve studies, algae between 2 and 3 months old (in lag phase growth) are inoculated into fresh WC medium

(Guillard 1978) and placed on a shaker table in a walk-in growth chamber which is set at a temperature and light/dark cycle dependent upon the experiment. Prior to introduction of the algae into the new medium with different growth conditions, the batch cultures are acclimated to the temperature and light regime for a period of two weeks to insure that results are not those of temperature or light shock. Aliquots are withdrawn in duplicate from thoroughly mixed 6 liter flasks 2-3 times a week for a period of approximately 6 weeks. Depending on cell density, between 75 and 200 ml are withdrawn per sample, filtered onto prewashed and preweighed Gelman A/E glass fiber filter, air dried, then dried in a vacuum at 60° for 24 hours. The filters are then reweighed to determine dry weight, and frozen for subsequent lipid extraction. Concurrent with dry weight analyses, smaller volumes of culture (9 ml) are withdrawn and placed in a tube containing paraformaldehyde-glutaraldehyde at final concentrations of 1% in 0.05 M sodium cacodylate buffer. Cell counts are performed on these samples with either a hemocytometer or plankton counting cell to determine cell densities.

For lipid analysis, the frozen filters are placed in a pre-extracted thimble and extracted in a micro-Soxhlet with chloroform for 12 hours (Orcutt and Patterson 1975). The extract is then concentrated in a Kuderna-Danish flask, evaporated under nitrogen, redissolved in chloroform and separated with a separatory funnel. This extract is dried under nitrogen stream in pre-weighed Teflon lined screw cap amber vials and weighed for

total gravimetric lipid. Samples are then flushed under nitrogen and frozen for analyses of the lipid classes.

For lipid class analysis, samples are redissolved in methylene chloride to concentrations of 20 to 50  $\mu\text{g}$  lipid in spotting volumes of 10 to 20  $\mu\text{L}$ . The samples are spotted with Hamilton syringes onto cleaned and blank scanned silica coated chromarods (type SIII), held in a frame and developed and scanned in an Iatroscan Mark IV (TLC-FID, FTID) system using a development system described by Parrish (1986).

The development is three staged, consisting of the following:

1. Developed in 50 ml solution of hexane, diethyl ether, and formic acid (99:1:0.05) for 25 minutes, conditioned for 5 minutes, and redeveloped in the same solvent system for 20 minutes. Rods are then partially scanned for hydrocarbons, wax esters, and ketones.
2. The rods are then reconditioned and developed in 50 ml of a solution of hexane, diethyl ether, and formic acid (80:20:0.1) for 40 minutes. The rods are then again partially scanned for triglycerides, alcohols, and sterols.
3. The third development consists of two 14 minute developments in 100% acetone, followed by two developments in 50 ml of solution containing dichloromethane, methanol, and water (5:4:1). During the last scan, the FTID detector is also used for the additional detection of N in Chla and phospholipids. The rods are scanned their entire

length for chlorophyll a, acetone-mobile polar lipids, and phospholipids.

Quantitative determinations of lipid class composition are based on dose-response calibration equations generated by analysis of a wide range of concentrations of standards for each lipid class.

To ensure quality control, standards are run with each set of samples on a frame (a frame holds 10 Chromarods). In addition, at all stages of analysis, samples are randomized. For example, in a study of growth and lipid content, all samples are collected for dry weight analysis and frozen. For gravimetric lipid content, samples from the experiment are randomly chosen from the freezer for Soxhlet extraction and these are usually run in groups of six. These extracts are kept frozen until the separatory funnel stage, and the samples are again randomized so that duplicate samples are not run on the same day at any stage. This is to avoid "holes" in the data set in case of a catastrophic event. Randomization also leads to longer analysis time because we may be extracting samples from several experiments simultaneously. However, we feel that in terms of the length of time involved in setting up the growth experiments, it is most prudent to analyze the samples in this fashion. The randomization is also extended to lipid class analysis on the Iatroscan in the same fashion.

## RESULTS

### 1. GROWTH EXPERIMENTS AND LIPID CONTENT AT 20°C.

#### A. 16:8 hr Light/Dark Cycle

The first growth experiments were conducted with the algae acclimated to 20°C and a light/dark cycle of 16:8 hr. These conditions approximate day length and surface water temperature in the Great Lakes at a latitude of 45°N during the summer. Under these conditions, growth was modest (Fig. 1). Aged Cyclotella cells had the highest total extractable lipid content (TEL) at the onset of the experiment. Lag phase is prolonged for roughly 2 weeks, and log growth is observed for approximately 1 week. During this time, TEL (as a percent of total dry weight) steadily declines, as was initially expected. Melosira grew with a different pattern. Modest growth was observed throughout the entire experiment, and TEL increased during the first week, and thereafter declined. Stephanodiscus experienced the least growth, and except for one week in culture, also had lower levels of TEL. The initial transfer, even though the cells had been acclimated to the temperature and light regime, resulted in a decline in living cells, and growth recovery was slow. However, when growth did resume at 23 days, TEL was substantially reduced.

Lipid class analysis demonstrates the composition of the TEL during the time period examined (Fig. 2). In Cyclotella, the TEL of aged cells (Day 0) is composed primarily of triglycerides, or storage lipids. Triglycerides decrease substantially by the second day in culture, and there is an increase in chlorophyll



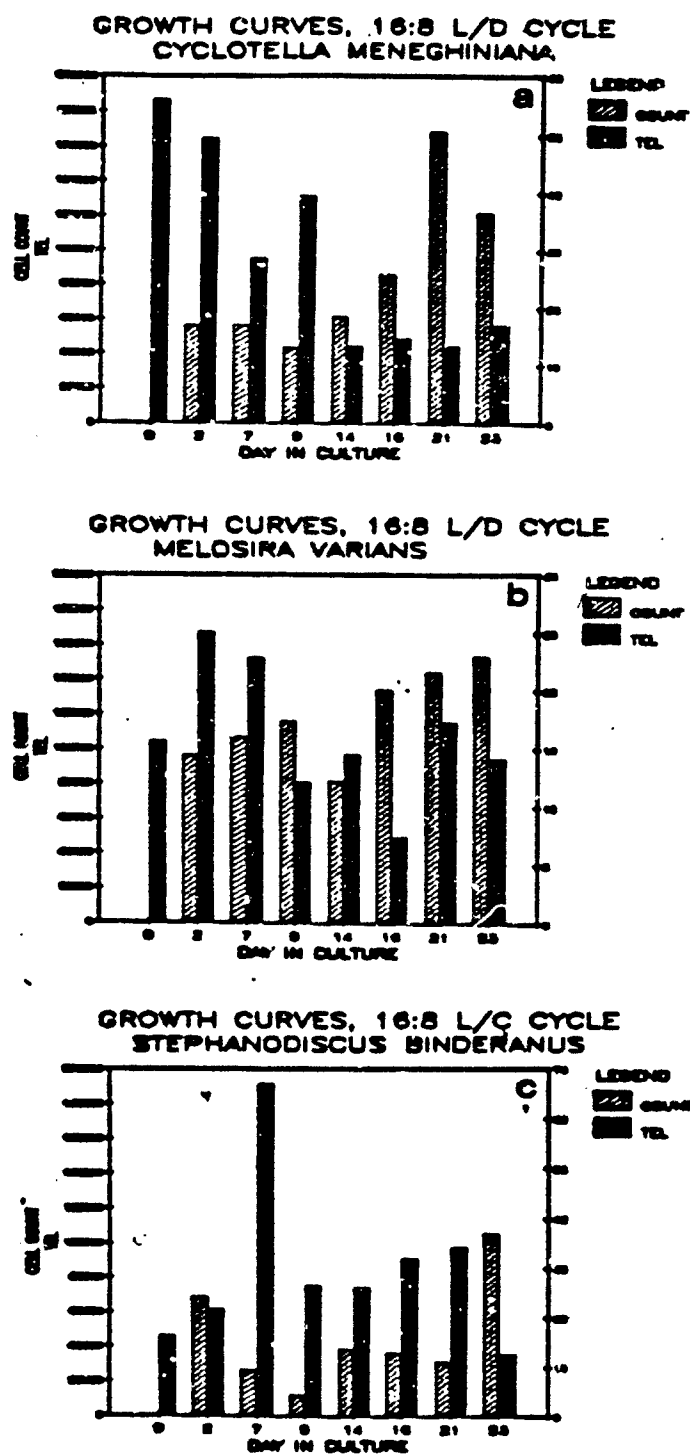
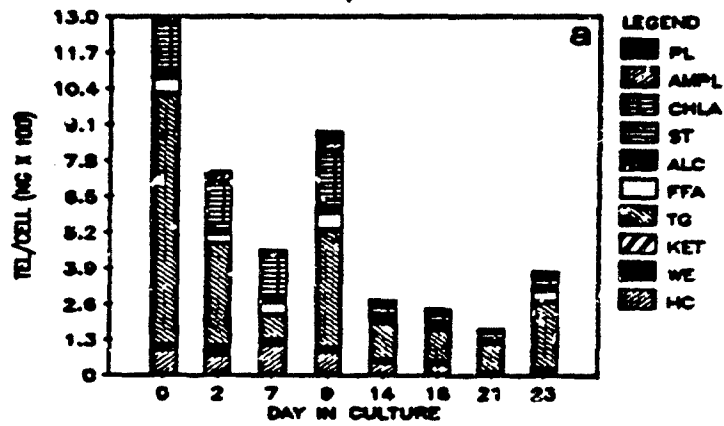


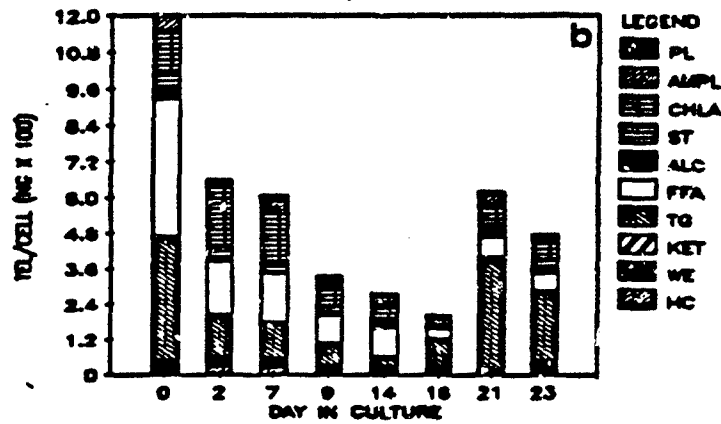
Figure 1. Growth curves for 16:8 L/D cycle at 20°C. Solid bars are total extractable lipid as a percent of total dry weight, hatched bars are all cell counts in numbers/mL.

- a. Cyclotella meneghiniana
- b. Melosira varians
- c. Stephanodiscus binderanus

**CYCLOTELLA MENECHINIANA  
LIPID COMPOSITION WITH GROWTH  
16:8 L/D CYCLE**



**MELOSIRA VARIANS  
LIPID COMPOSITION WITH GROWTH  
16:8 L/D CYCLE**



**STEPHANODISCUS BINDERANUS  
LIPID COMPOSITION WITH GROWTH  
16:8 L/D CYCLE**

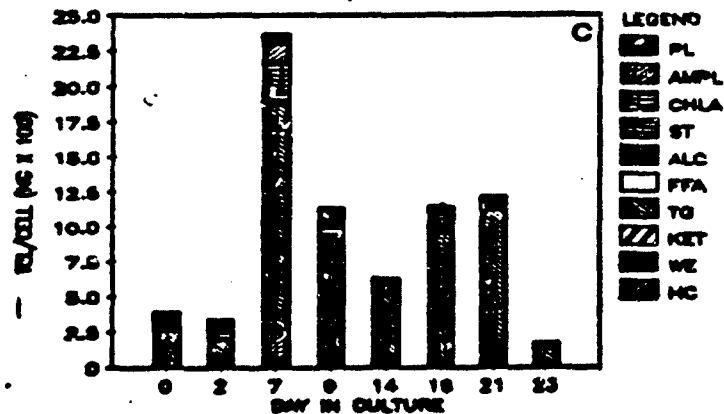


Figure 2. Stacked bar chart of lipid composition with growth at 20°C on a 16:8 L/D cycle. Height of stacked bar is TEL; composition is indicated by patterns designated on the right.

- a. Cyclotella meneghiniana
- b. Melosira varians
- c. Stephanodiscus binderanus

and phospholipids before growth commences on Day 14. Rapid growth is characterized by rather steady percentages of triglycerides and chlorophyll (Fig. 2A), followed by an increase in triglycerides and a decrease in chlorophyll a as growth decreases.

Lipid class composition of Melosira varians is substantially different from that of Cyclotella (cf. Figs. 2A, 2B). While Cyclotella has a relatively small percentage of free fatty acids, they are one of the most prevalent classes found in Melosira. Although they were once believed to be an analysis artefact, it has been shown recently (Parrish 1986) that they may constitute up to 25% of the total lipids in certain phytoplankton. The highest concentration of free fatty acids found in Melosira was approximately 25% on Day 0. Percentages were reduced as growth commenced. However, they remain a significant portion of the total lipid composition. An increase in Chlorophyll and a reduction in triglyceride is observed with growth, in a pattern analogous to that observed in Cyclotella. The increase in phospholipids on day 7 is also similar. As growth continues, the total lipid pool decreases.

Growth of Stephanodiscus under the conditions described above is marked by very slow growth. In fact, transfer to new medium actually led to a dramatic decline in numbers of living cells within one week. This reduction in numbers was accompanied by an extensive increase in TEL, with the most marked change occurring in the triglycerides. Lipid class composition in Stephanodiscus (Fig. 2C) is also marked by an increase in sterols

on the 7th day in new medium and is accompanied by an increase in chlorophyll. Growth recovers at 23 days and this period is marked by a substantial reduction in TEL, with the predominant lipid classes being triglycerides and phospholipids. Detailed changes in lipid classes with growth for all three organisms are detailed in Figs. 3-5.

#### B. 12:12 hr Light/Dark Cycle at 20°C

As previously mentioned, all experiments were initiated after a two week acclimation of lag phase cultures to the new light regime. This two week acclimation led to a total demise of Cyclotella meneghiniana, consequently data are only presented for Melosira and Stephanodiscus.

Both organisms grew well on a 12:12 hr L/D regime and present a more classical case of a sigmoidal growth curve (Fig. 6). Melosira went through a lag period for approximately two weeks before growth increased rapidly (Fig. 6A). Growth was sustained for approximately two weeks, then the cells began stationary phase growth. The pattern is similar in Stephanodiscus (Fig. 6B) except that the cells take approximately a week longer to begin log and stationary growth. Two to three times the cell densities were observed in this light regime compared with the 16:8 hr L/D cycle.

Total extractable lipid (TEL) also follows a similar pattern. High lipid content is observed in cells before active growth commences. Lipid content is low just before active growth

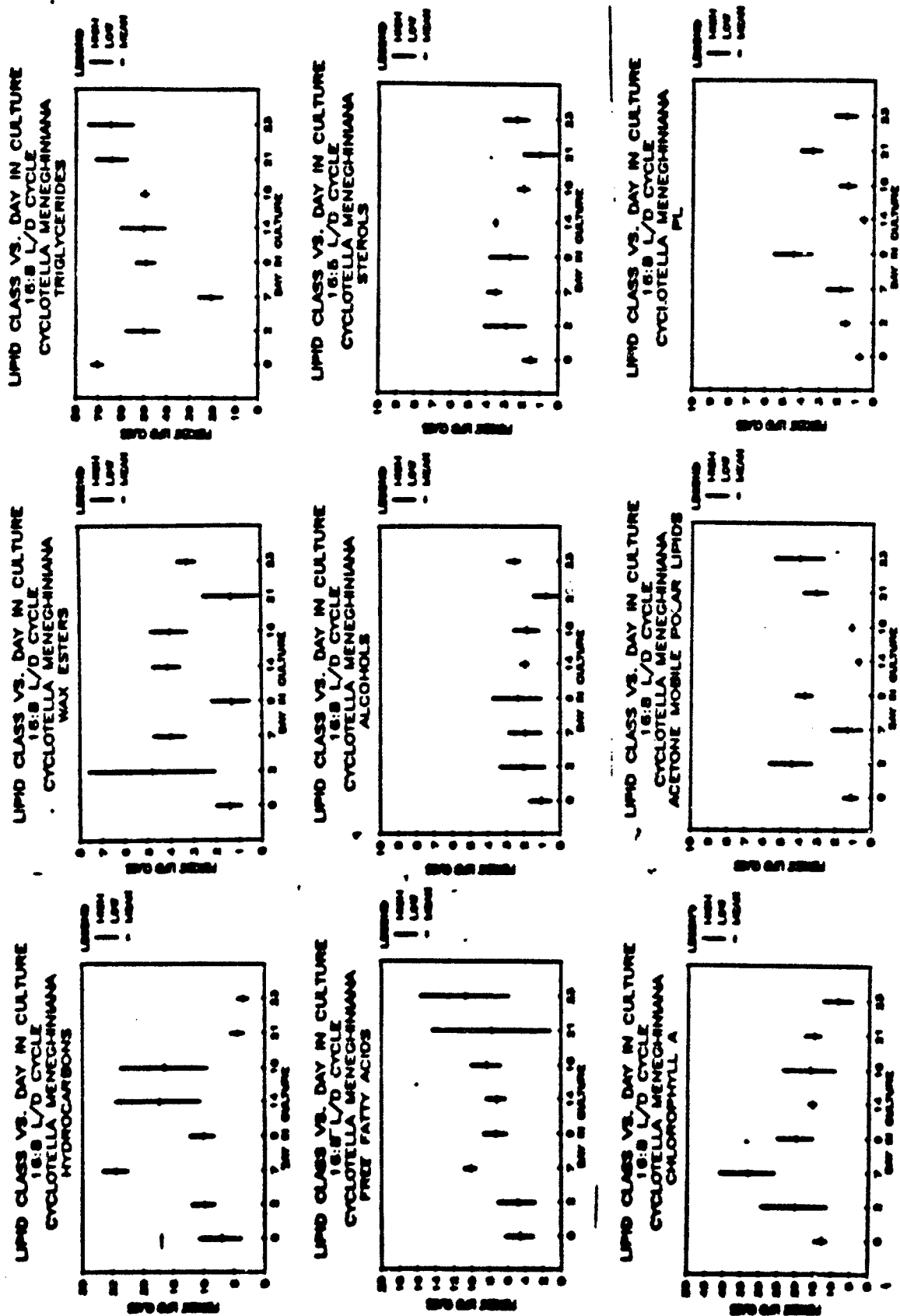


Figure 3. Detailed view of lipid classes with growth for *Cyclotella meneghiniana*. Numbers reported are the percent of total lipid  $\pm$  1 S.E.

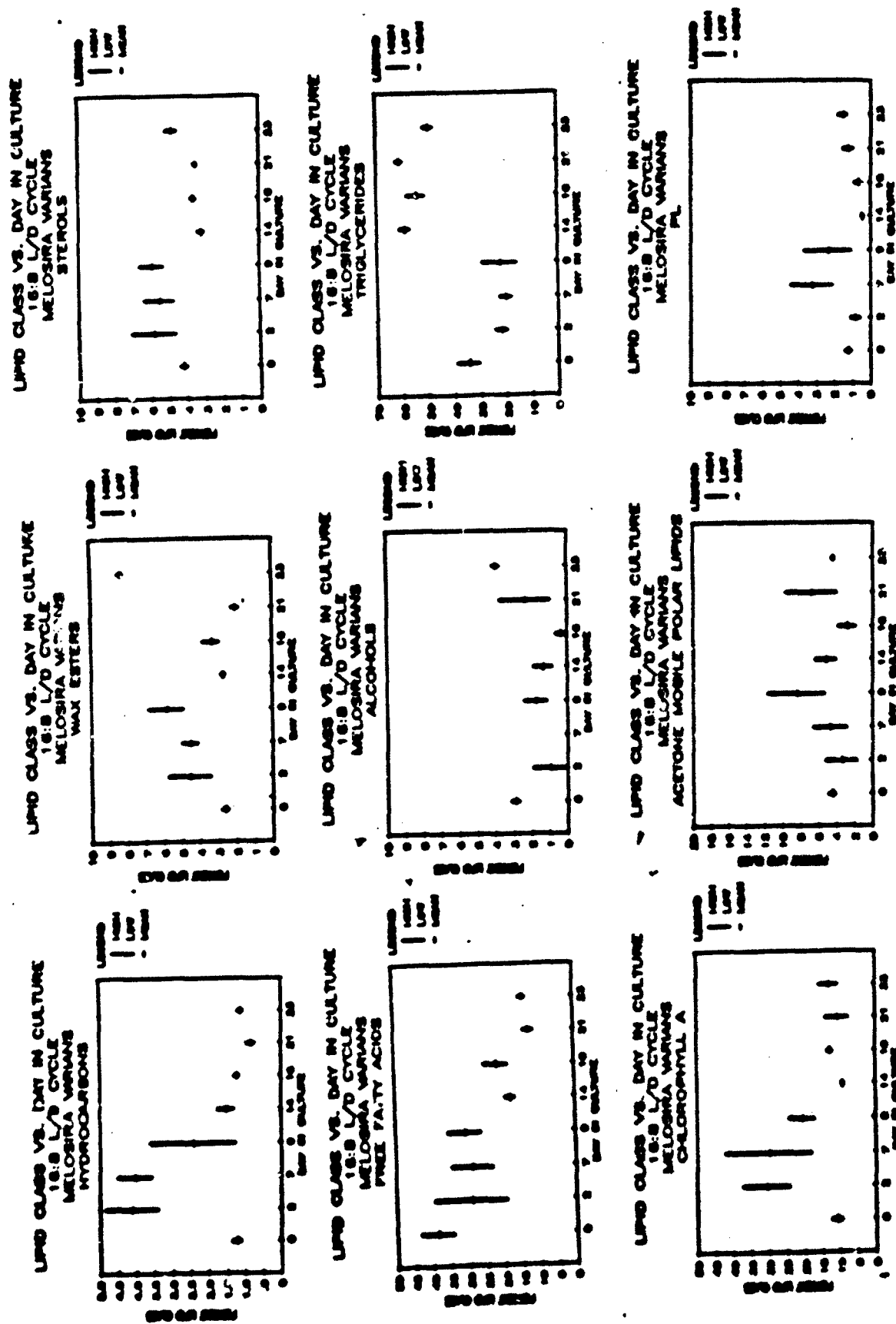


Figure 4. Detailed view of lipid classes with growth for *Melosira varians*. Numbers reported are the percent of total lipid  $\pm$  1 S.E.

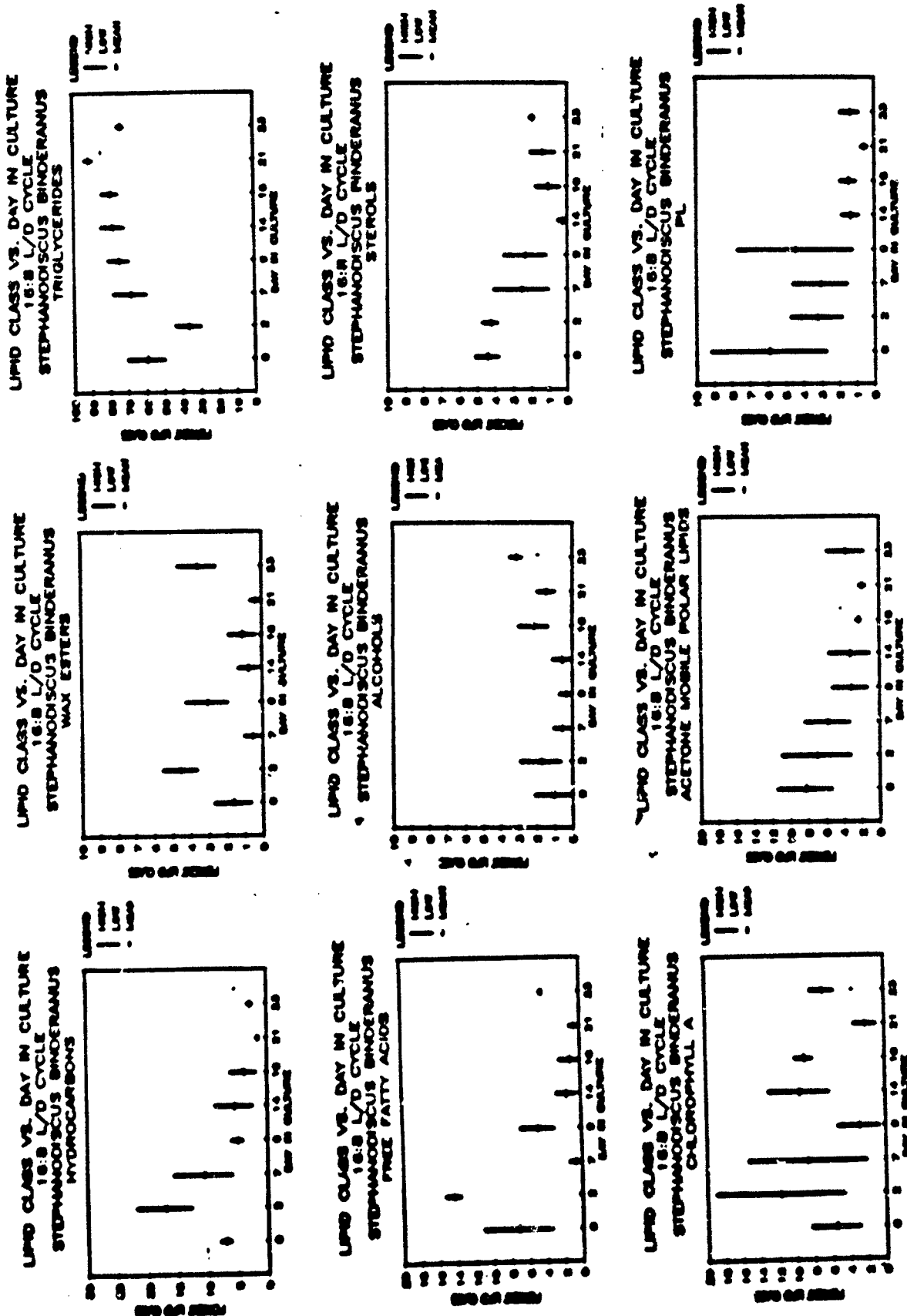
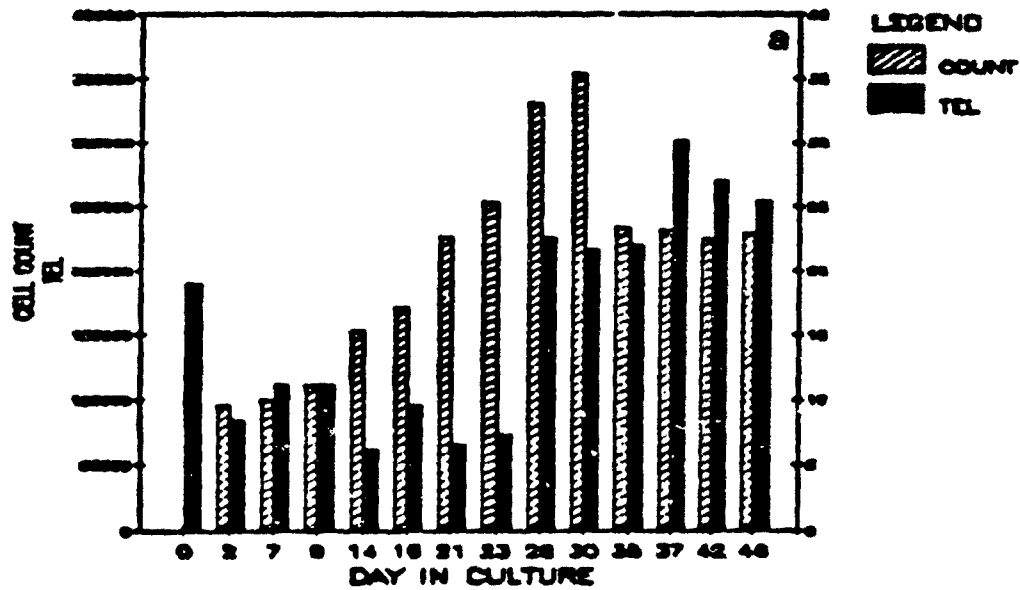


Figure 5. Detailed view of lipid classes with growth for *Stephanodiscus binderanus*. Numbers reported are the percent of total lipid  $\pm$  1 S.E.

# GROWTH CURVES, 12:12 L/D CYCLE MELOSIRA VARIANS



# GROWTH CURVES, 12:12 L/D CYCLE STEPHANODISCUS BINDERANUS

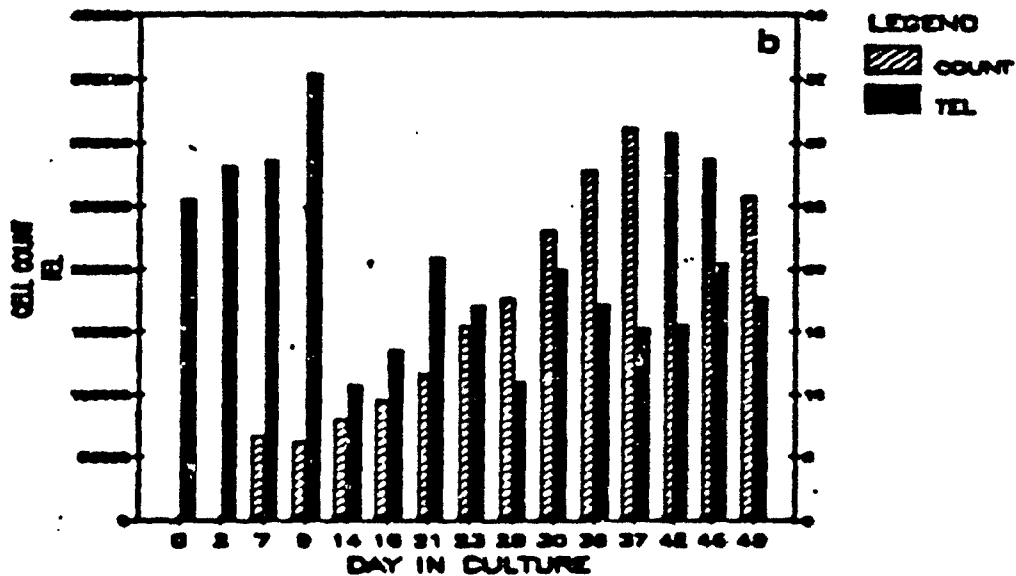


Figure 6. Growth curves and TEL data for cells grown on a 12:12 hr L/D cycle at 20°C.

a. *Melosira varians*

b. *Stephanodiscus binderanus*



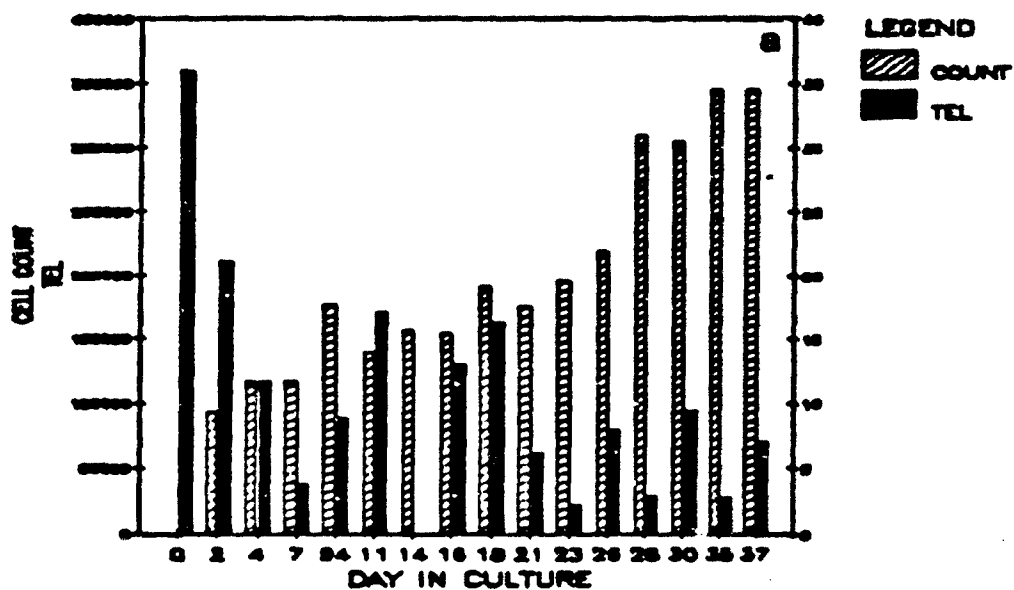
begins, and increases as cells age. Detailed lipid class analysis is incomplete, so no data are presented at this time.

#### C. 20:4 hr Light/Dark Cycle at 20°C

Just as an aberrant growth pattern was observed with the 12:12 hr L/D cycle, another anomaly was observed with the 20:4 hr L/D cycle. During this acclimation period, Stephanodiscus binderanus became non-viable and would not thrive. Consequently, data are only present for Cyclotella and Melosira (Fig. 7). Cyclotella experienced sustained growth at the longer day lengths (Fig. 7A). TEL is initially very high, is reduced at seven days in cultures and is found at minimal levels throughout the remainder of the sampling period. Cell numbers steadily increased and never exhibited a reduction in approximately 7 weeks of sampling. One experiment (the first time we ran the 16:8 hr L/D cycle) was inadvertently set up with the timers on the growth chamber misadjusted, and the lights were on continuously for 24 hours. Cyclotella achieved its highest doubling rate under these conditions of continuous light.

Although Melosira grew at the longer day length, it sustained rapid growth for only a period of one week, then steadily declined (Fig. 7B). TEL was initially high, followed by a reduction before rapid growth, then a steady decrease for the remainder of the sampling period. Lipid class analysis for this experiment is also incomplete at this time.

# GROWTH CURVES, 20:4 L/D CYCLE CYCLOTELLA MENECHINIANA



# GROWTH CURVES, 20:4 L/D CYCLE MELOSIRA VARIANS

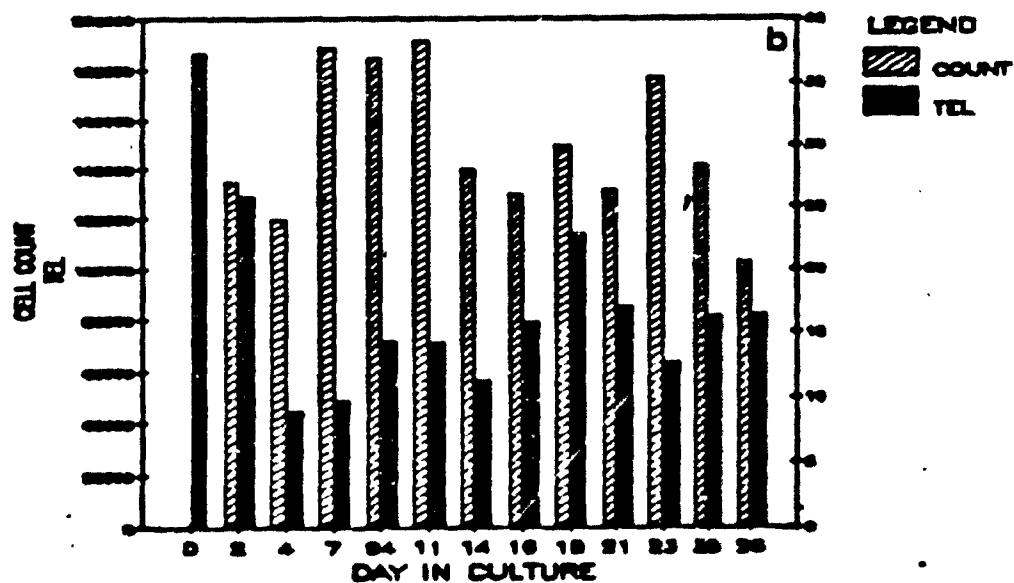


Figure 7. Growth curves and TEL data for cells grown at 20°C on a 20:4 hr L/D cycle.

- a. *Cyclotella meneghiniana*
- b. *Melosira varians*

The observations about what appeared to be a light cycle preference have been and are again going to be examined in more detail. We repeated growth curve determinations under very controlled conditions with equal inocula for all three organisms under the three light regimes described above. Preliminary results suggest that there is a strong preference by two of the organisms, Cyclotella and Stephanodiscus for particular day length durations. These experiments will be repeated again after the first of the year since there is little discussion, if any, concerning the role of light cycle in phytoplankton population dynamics in the lakes. Current theory is that the predominant factors controlling the diatom bloom in the Great Lakes in early spring and late fall is a combination of water temperature and high dissolved silica levels. Our results suggest that all other factors being constant, day length can control population dynamics. Consequently we will repeat this experiment again before the data is prepared for publication. In considering the natural distribution of these organisms, the results are not actually surprising. Stephanodiscus is normally an early spring-late fall dominant, when day length is roughly 12 hours in duration at our latitude. Cyclotella is normally more dominant in the summer, with longer day lengths, and Melosira can be found in the spring bloom, but is also a minor component of the phytoplankton assemblage throughout the year.

#### D. Changes in Lipid Content over a 24 hr Period:

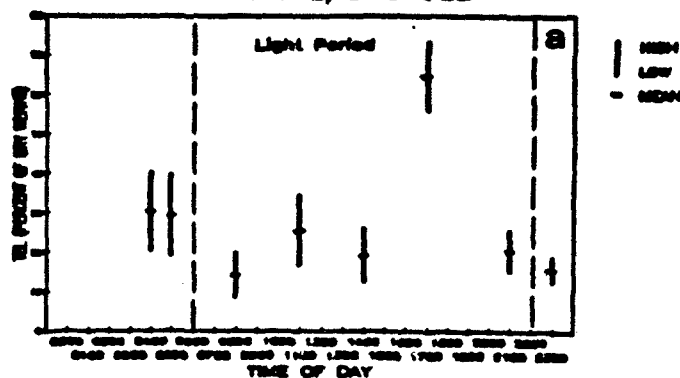
##### 16:8, 12:12, and 20:4 hr Light/Dark Cycles

Concomitant with determinations of total lipid during a growth cycle, studies were conducted with each of the experimental diatoms in logarithmic growth to determine the fluctuations in lipid content and composition during a 24 hour period. These studies were performed for all light cycles studied. It should be noted here that for growth cycle studies, all samples were taken at the same point in the light/dark cycle, i.e., cultures were sampled at the fourth hour of the light period.

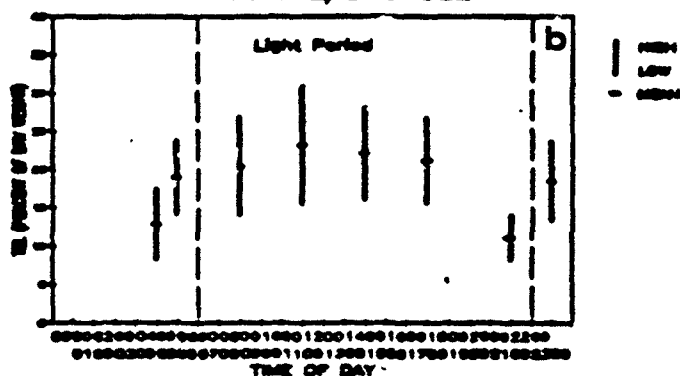
##### 1. 16:8 hr L/D Cycle

The lipid content of all organisms examined varies greatly during a 24 hour period. However, several aspects of the lipid content appear to be similar. First, at the end of the dark period, all organisms have rather steady values for lipid content and the ranges suggest that the numbers may be quite similar. The value at the onset of the dark period is also within this range. The greatest reduction in lipid content occurs in all three organisms just prior to the onset of the dark period. This reduction, in all cases, is preceded by a generally high lipid content. Although analysis of lipid composition is incomplete, the data suggests that the initial increase is due to the increase in chlorophyll content. The timing of the decrease in total lipid is most similar for Melosira and Stephanodiscus (Fig. 8).

LIPID VS. TIME OF DAY  
CYCLOTELLA MENEHINIANA  
16:8 L/D CYCLE



LIPID VS. TIME OF DAY  
MELOSIRA VARIANS  
16:8 L/D CYCLE



LIPID VS. TIME OF DAY  
STEPHANODISCUS BINDERANUS  
16:8 L/D CYCLE

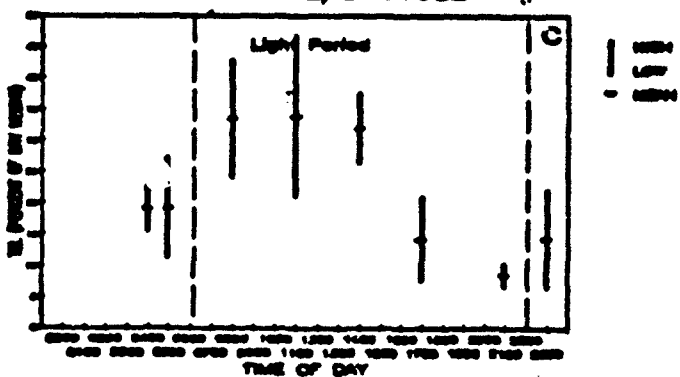


Figure 8. Lipid variation on a 24 hour basis. The light period is marked by broken lines. 16:8 hr L/D cycle at 20°C.

- a. Cyclotella meneghiniana
- b. Melosira varians
- c. Stephanodiscus binderanus

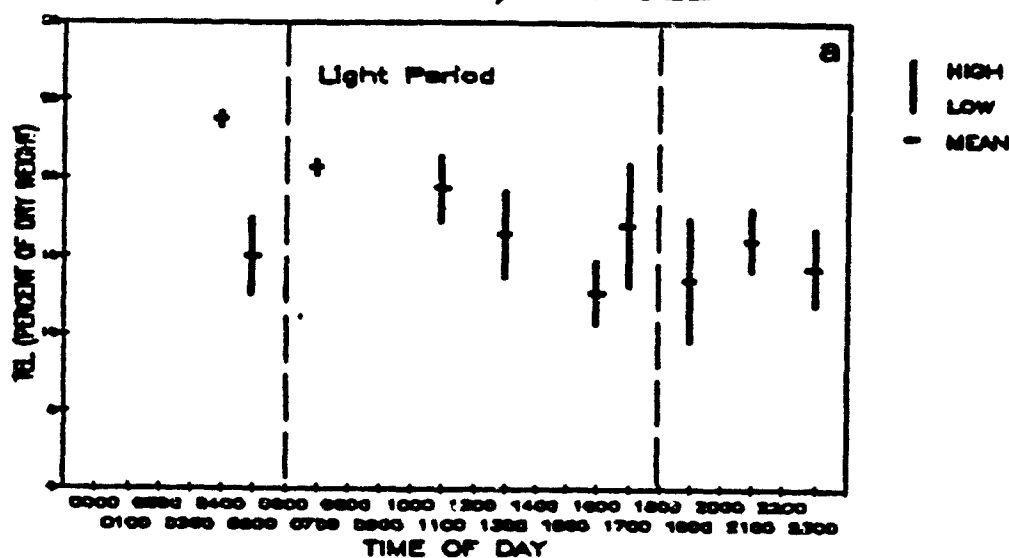
## 2. 12:12 hr L/D Cycle

Lipid content in Melosira and Stephanodiscus appears to be more cyclical under a 12:12 hr L/D regime (Fig. 9A). Melosira appears to have a higher lipid content prior to the onset of the light period, followed by a reduction in total lipid just before the lights come on. Concomitant with the initiation of the light period, there is an increase in lipid content, followed by a decline to nearly steady levels at the onset of the dark. In Stephanodiscus (Fig. 9B), the lipid content is quite cyclical. The large increase at the onset of the light period is followed by reductions mid-cycle, and a decrease before dark onset. The data suggests that throughout the dark period lipid content varies considerably, with noticeable reductions at the onset of the dark period followed by an increase.

## 3. 20:4 hr L/D Cycle

The lipid content pattern also changes with the 20:4 hr L/D cycle (Fig. 10). Lipid content in Cyclotella increases slightly after the dark period, but the most significant increase occurs twelve hours into the light period. Lows in lipid content are observed four hours before the onset of the dark period and six hours after the onset of the light period. The pattern in Mclosira is somewhat similar to that observed with the other light regimes, although lipid content is significantly lower.

LIPID VS. TIME OF DAY  
MELOSIRA VARIANS  
12:12 L/D CYCLE



LIPID VS. TIME OF DAY  
STEPHANODISCUS BINDERANUS  
12:12 L/D CYCLE

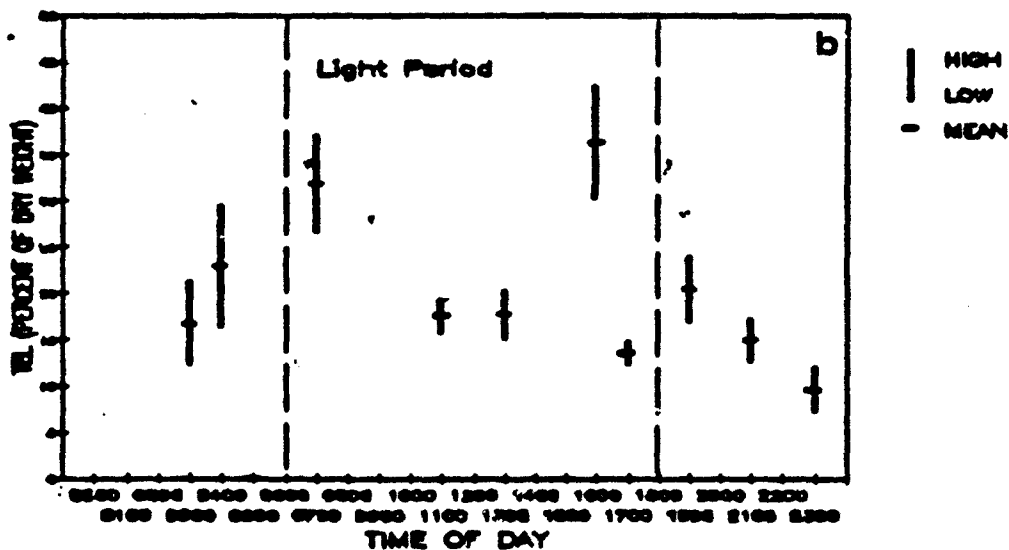
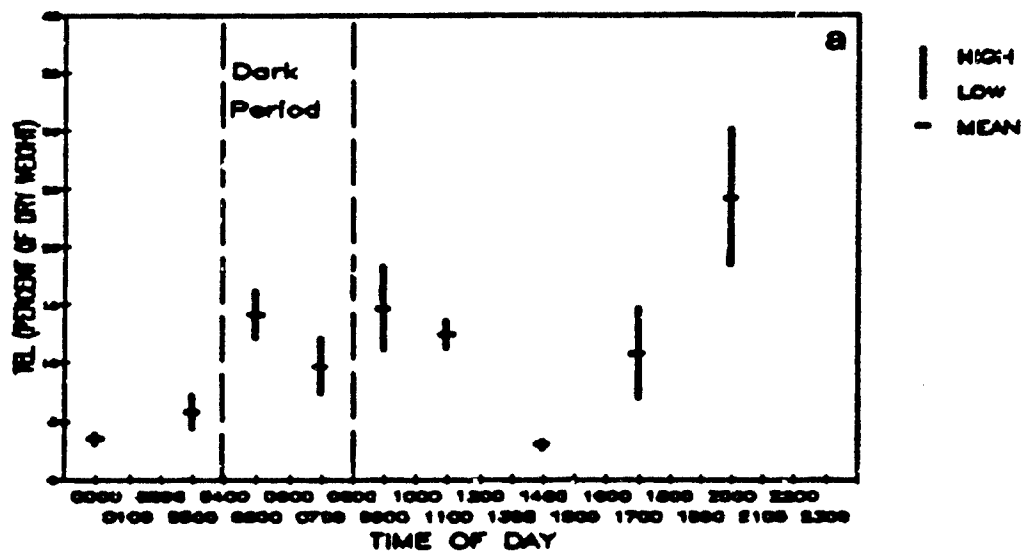


Figure 9. Lipid variation during a 24 hour period. The light period is designated by broken lines perpendicular to the X-axis. 12:12 hr L/D cycle at 20°C.

- a. Melosira varians
- b. Stephanodiscus binderanus

LIPID VS. TIME OF DAY  
CYCLOTELLA MENEGHINIANA  
20:4 L/D CYCLE



LIPID VS. TIME OF DAY  
MELOSIRA VARIANS  
20:4 L/D CYCLE

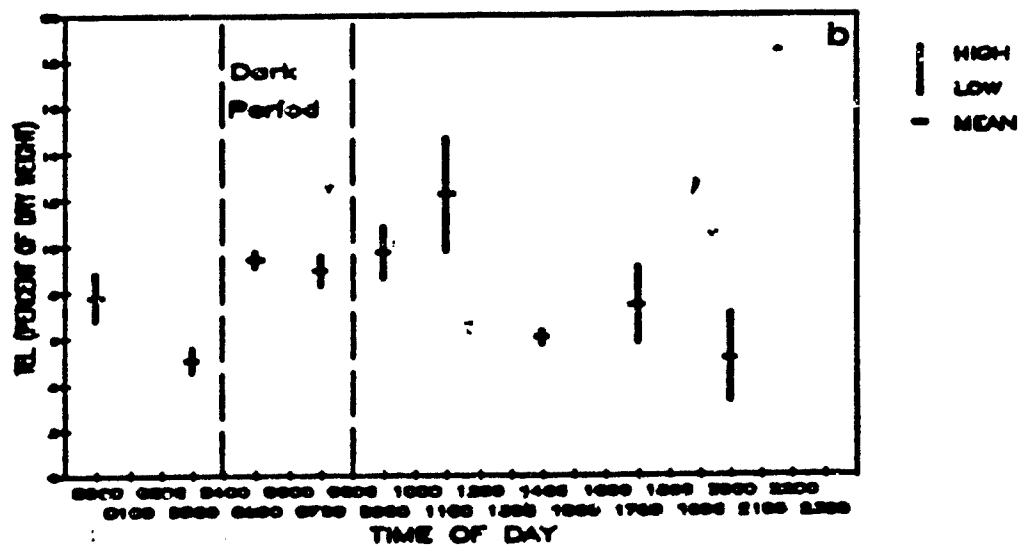


Figure 10. Lipid variation during a 24 hour period. The dark period (4 hrs) is designated by broken lines perpendicular to the X-axis.

- a. Cyclotella meneghiniana  
b. Melosira varians



#### 4. Comparison of Changes in Lipid Content by Organism

When changes in total extractable lipid over a 24 hour period are plotted for each organism (Fig. 11), one clear pattern emerges. TEL reaches a maximum during the time between mid- to late light cycles, and this maximum is consistently followed by a sharp drop in TEL just prior to the onset of the dark period. For example, in Cyclotella meneghiniana, cells grown on a 16:8 hr L/D cycle reach their highest lipid content at 5 pm, and this is followed by a drastic reduction at 9 pm (Fig. 11A). Similarly, cells grown on a 20:4 hr L/D cycle have highest TEL values at 8 pm, followed by a very low value at midnight, again 4 hours later. This pattern is similar, especially for the 16:8 hr L/D cycle in Melosira (Fig. 11B), and is less pronounced, though regular, with the other light/dark cycles. Stephanodiscus binderanus (Fig. 11C) also exhibits this periodicity for the two light/dark cycles examined.

This periodicity is being further studied in the same organisms under the same light regimes, but at 15°C. The 24 hour sampling on the 16:8 hr L/D cycle is complete, and samples are presently being extracted for TEL. 24 hour sampling of the 20:4 hr L/D cycle is due to begin the week of January 8th. In addition to the data collection we have done or are in the process of completing, I submitted a small project description to the Women in Science Program at the University for an independent Undergraduate Research Internship Program for a student to investigate this phenomenon in more detail. I was assigned as a non-

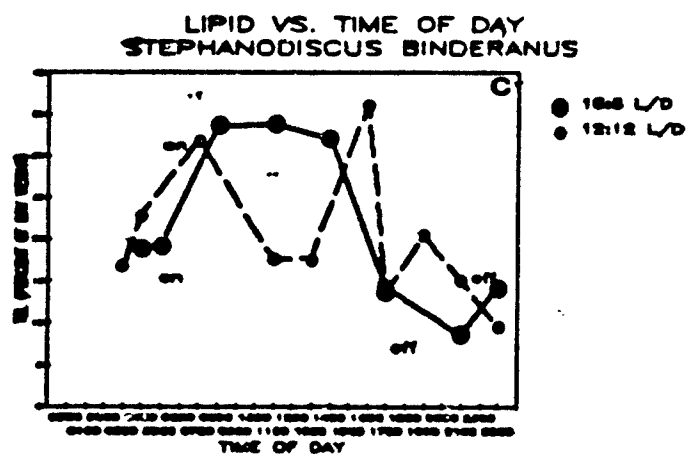
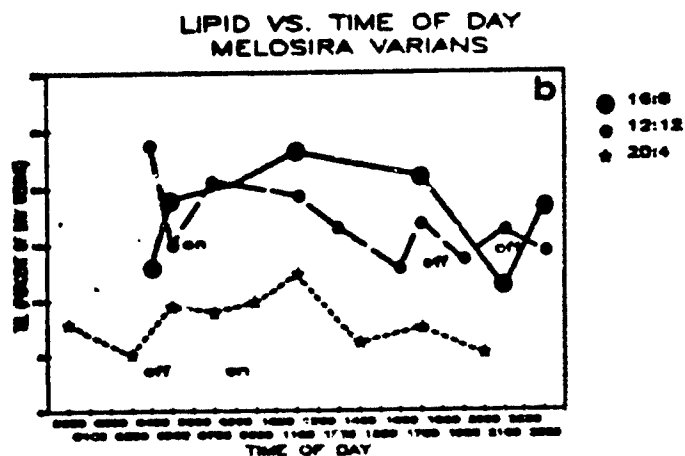
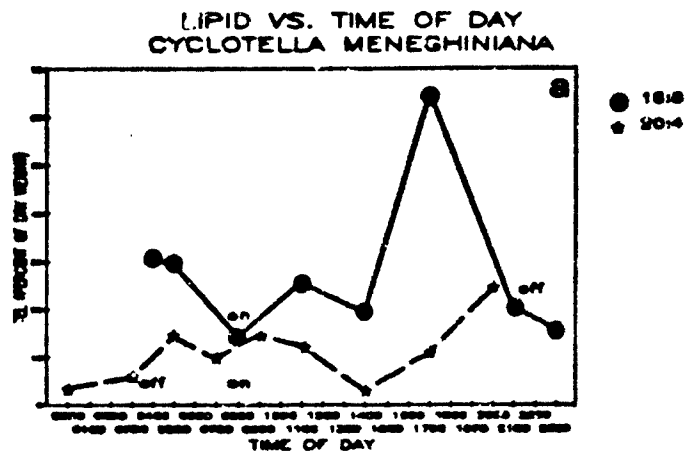


Figure 11. Comparison of changes in total extractable lipid (%T.E.) over a 24 hour period for all light regimes.

- a. Cyclotella meneghiniana
- b. Melosira varians
- c. Stephanodiscus binderanus

tor to Ms. Sabrina Henry, an undergraduate Native American student, who will run additional experiments this winter semester to investigate if the phenomenon is totally light dependent, or if the phenomenon may be of an ultradian nature, i.e. occurring at times independent of the light cycle. Ms. Henry will determine the TEL for 24 hour periods in continuous light and in continuous darkness to determine the dependency on light entrainment. This position is independently funded by the University, but the research will be conducted in conjunction with our ongoing studies.

#### EFFECT OF LIPID CONTENT ON SUSCEPTIBILITY TO LIPOPHILIC TOXICANTS

After it was determined that lipid content varied considerably during a 24 hour period, a preliminary experiment was performed to determine if lipid content varied with exposure to chlorinated hydrocarbons. For the experiment, two organisms were tested; Cyclotella meneghiniana and Melosira varians. Cells were exposed to 0.245 ppm 1,3,5-trichlorobenzene in a closed system and sampled at 0, 2, 24, and 48 hrs of exposure to the toxicant. One exposure was initiated during the 11th hour of the light period, and the second exposure was initiated during the 15th hour of the light period. Preliminary data suggested that at the 11th hour of the light cycle (cf. Figs. 11A and 11B, time = 1700), lipid content in both organisms was high, and at the 15th hour of the light cycle (time = 2200), lipid content was low.

For convenience of sampling (and safety of the technicians), the growth chamber was reset so that the lights came on at 3 am, five hours earlier than previously used in the 16:8 hr L/D cycle studies. Results of the total lipid extraction are presented in Figures 12 and 13. In Cyclotella meneghiniana (Fig. 12), lipid content indeed was different at 11 and 15 hours into the light cycle. However, the values were the reverse of what was predicted. That is, the starting values at the 11th hour were low, and values for the 15th hour were high. There was an increase in lipid content after 24 hours in cells exposed to 1,3,5-trichlorobenzene in the 11th hour of the light cycle. This appears to be the only significant change in lipid content. Figure 13 shows the data for Melosira varians. The initial lipid content values were again, unexpected and not what were predicted. Values were approximately the same at 11 and 15 hours into the light cycle. Exposure to the chlorinated benzene resulted in a significant increase in lipid content after 2 hours in cells exposed during the 11th hour of the light cycle. Detailed lipid analyses are as yet incomplete.

TEL data were examined more closely in relationship to the 24 hour study, and it has been determined that the pattern observed in total lipid content approximates the trends observed in the 24 hour sampling in relationship to absolute time. That is, if the data in Fig. 11A are examined in relationship to time of day, the lowest lipid content occurred at 2 pm in both the 16:8 and 20:4 hr L/D cycles, and higher values occurred at 6 pm, irre-

# CYCLOTELLA MENECHINIANA 1,3,5 TRICHLOROBENZENE EXPOSURE LIGHT CYCLE DEPENDENT RESPONSE

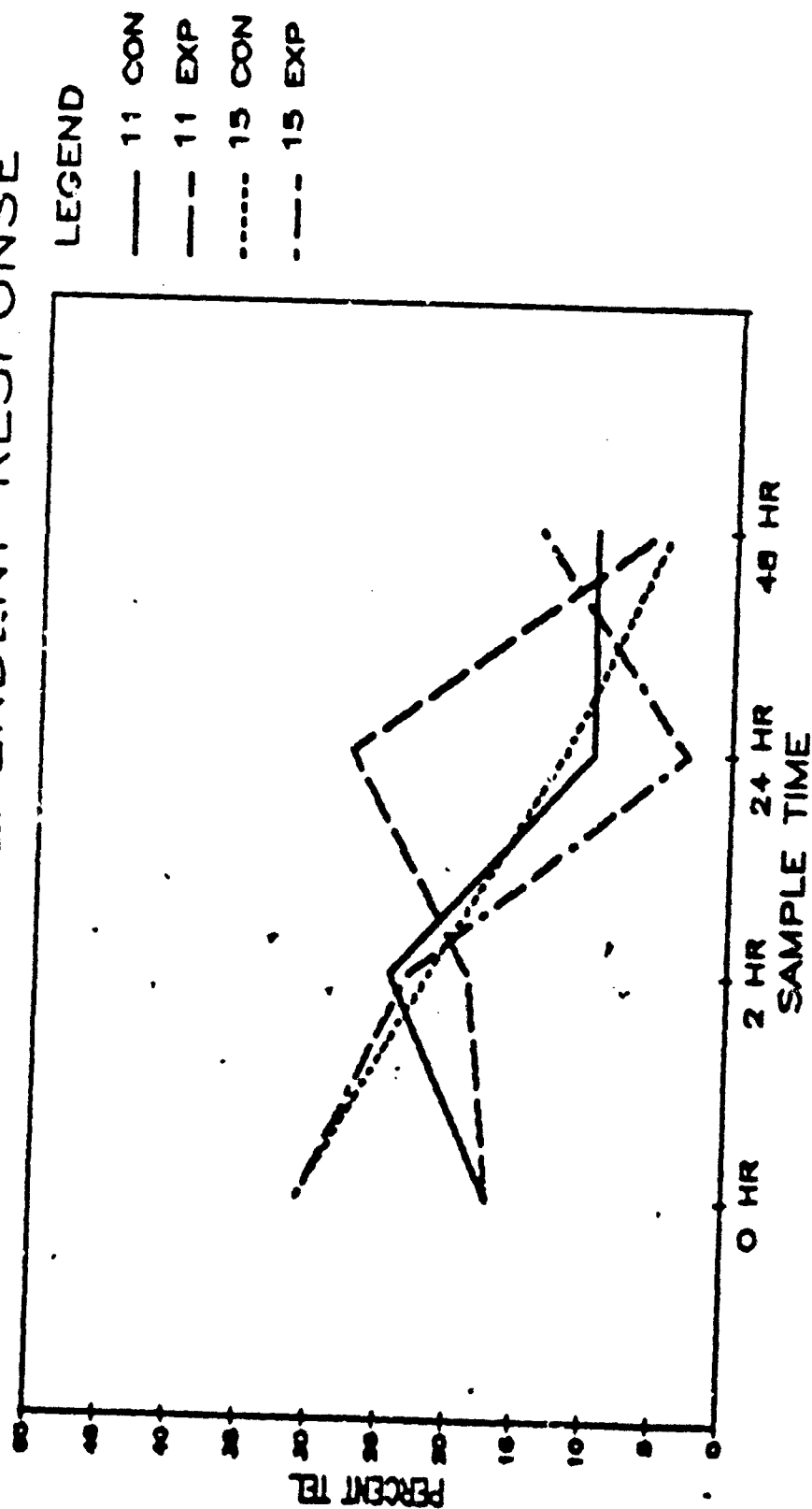


Figure 12. Total lipid content as a function of exposure to 1,3,5-trichlorobenzene (0.245 ppm). 11,15 CON = control cultures. 11 EXP = cells exposed to 1,3,5-trichlorobenzene where initial exposure occurred at the 11th hour of the light cycle on a 16:8 hr L/D regime. 15 EXP = cells exposed to 1,3,5-trichlorobenzene where initial exposure occurred at the 15th hour of the light cycle on a 16:8 hr L/D regime.

# MELOSIRA VARIANS 1,3,5 TRICHLOROBENZENE EXPOSURE LIGHT CYCLE DEPENDENT RESPONSE

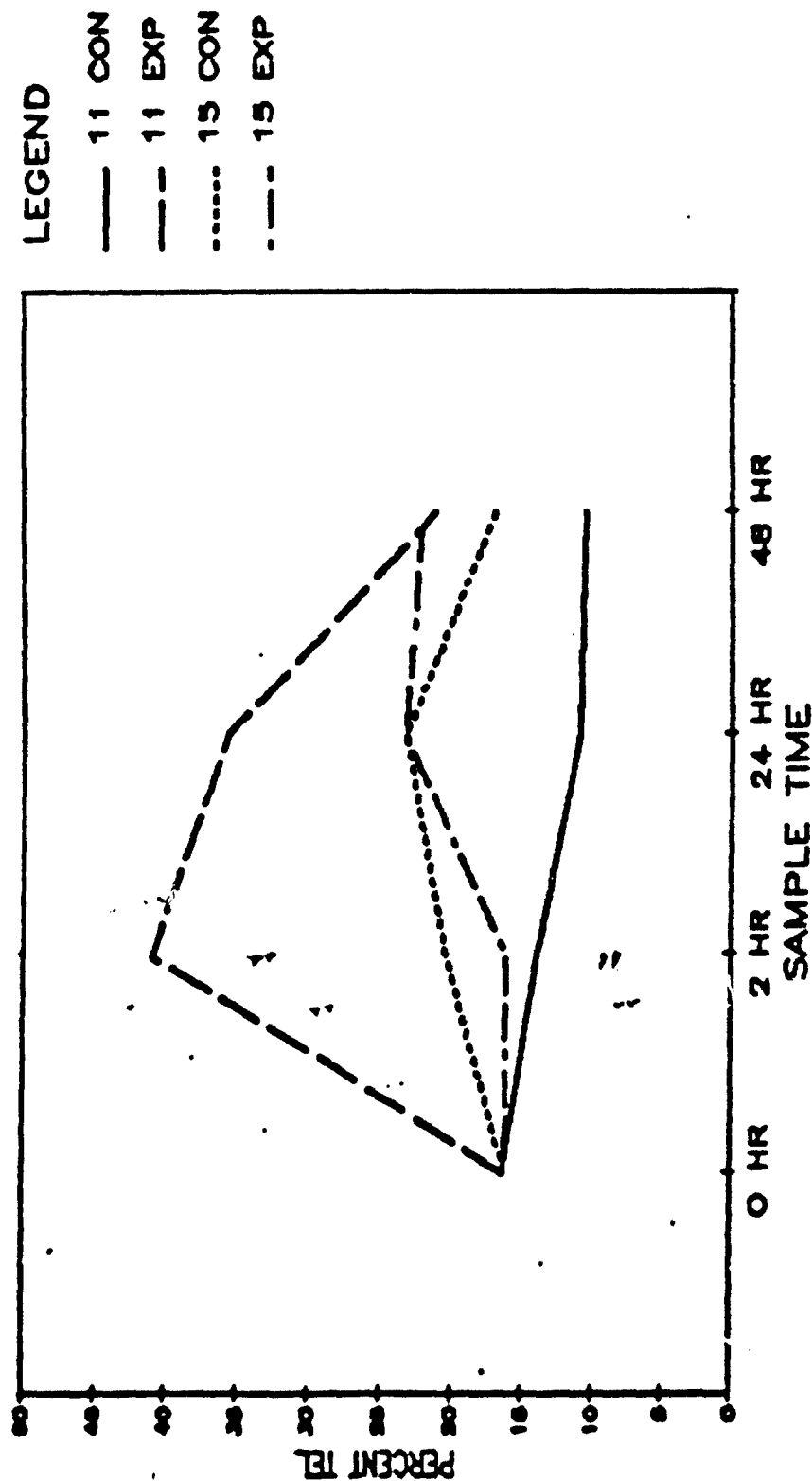


Figure 13. Total lipid content as a function of exposure to 1,3,5-trichlorobenzene (0.245 ppm). 11,15 CON = control cultures. 11 EXP = cells exposed to 1,3,5,-trichlorobenzene where initial exposure occurred at the 11th hour of the light cycle on a 16:8 hr L/D regime. 15 EXP = cells exposed to 1,3,5,-trichlorobenzene where initial exposure occurred at the 15th hour of the light cycle on a 16:8 hr L/D regime.

spective of the light cycle. Similarly, if one examines the data for Melosira (Fig. 11B), there is little difference in lipid content between samples taken at 2 pm and 6 pm for all light regimes. This data further suggests that lipid content may not be controlled completely by light cycle entrainment, and we plan to investigate this phenomenon in more detail.

#### PUBLICATIONS

The following papers are being prepared for publication:

1. Effect of the light/dark cycle on diatom lipid quantity and composition.
2. Effect of temperature on diatom lipid quantity and composition.
3. Intrinsic patterns of lipid production in diatoms.

These articles will most likely be submitted to the Journal of Phycology and the Journal of Plankton Research. Data reduction is nearly complete for the first two articles. Several experiments (constant daylight and constant darkness) need to be run to complete the third paper.

Authorship has not yet been determined. However, my policy is to include students and technicians who have been involved in data collection and reduction as coauthors.

## PARTICIPATING PERSONNEL

The following individuals, in addition to the P.I. are currently involved with the project:

- a. Dr. Norman A. Andresen. Dr. Andresen took over in November as Lab Manager. This position was formerly held by Ms. Rene Dillon, who left in November to more actively pursue her Master's Degree and a teaching assistantship at Eastern Michigan University. Dr. Andresen's specialty is phytoplankton, particularly diatoms, and he has also taught Plant Physiology.
- b. Ms. Anastasia Tabor. Ms. Tabor is currently a Master's student in the School of Natural Resources in the Aquatic Resources Program. Ms. Tabor is a first year graduate student who hopes to pursue a research topic relevant to lipids and phytoplankton. She is currently enrolled as a full-time student and the project is paying a portion of a stipend as a Graduate Student Research Assistantship.
- c. Ms. Yvonne Shih. Ms. Shih is a graduate student in the School of Public Health and she currently works part-time as a research assistant.
- d. Ms. Maria Jessena is a graduate student in analytical chemistry at the University of Wisconsin-Madison. Ms. Jessena has worked casually in the laboratory in November and December when she has been in the area visiting family. She most recently worked three full weeks during the Christmas period (she was in town visiting between



semesters) providing additional training to Dr. Andresen and Ms. Tabor.

#### INTERACTIONS

Two papers have been presented to date on results of the research pursued in this project. These are:

1. An invited seminar to the Biology Department, Wayne State University, Detroit Michigan, Winter Term 1989. Lecture entitled "Phytoplankton physiological adaptations and survival in large lake ecosystems."
2. An oral presentation at the Michigan Electron Microscopy Forum meeting in May 1989 in Kalamazoo, Michigan, titled "Lipid Production and accumulation in diatoms."

Poster presentations are being prepared for meetings of the American Society of Limnology and Oceanography (June) and the Phycological Society of America (June) in 1990.

#### ADDITIONAL STATEMENT

The first portion of the project has been devoted to determining normal patterns of lipid cycling in diatoms. The results are somewhat consistent with what was initially predicted, i.e. lipid content is higher in older organisms, and total lipids vary more over a period of 24 hours than they do over a period of weeks. What was unexpected is that the data suggest that some of

the lipid content variation is not entrained with the light cycle, the major factor that controls metabolism in autotrophic organisms. Additional experiments have been and are being added to verify this further before publication. The other data set that was unexpected, but not entirely a surprise, considering ecological distribution, was the growth-no growth behavior with regard to certain light regimes. Again, this topic has not been adequately addressed in the literature, so we are proceeding cautiously, repeating the experiments before publication. Although both of these observations have extended the scope of the project, the results have wide-ranging ecological implications in that light regime may do more to control phytoplankton distribution than previously realized and in that phytoplankton lipid content may have inherent variations that are not totally a result of light entrainment.